Studies on Ceylon Hæmatozoa.

No. II.—Notes on the Life-Cycle of Hæmogregarina nicoriæ, Cast. and Willey.

By

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With Plates 32–41 and 1 Text-figure.

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In 1904 Drs. Castellani and Willey (**3**) described a hæmogregarine from the blood of the common lake-tortoise of Ceylon, Nicoria trijuga. They named the parasite Hæmogregarina nicoriæ after its host. Shortly afterwards these authors gave a somewhat fuller account of their observations in the 'Quarterly Journal of Microscopical Science' (**4**).

While in Ceylon in 1907–08 I was able, largely through the kindness of Dr. Willey, to collect the material described in the following pages. My observations agree in the main with those of the earlier observers already cited. I have, however, been able to supplement their results and to give an account of some of the processes which take place in the intermediate host, the leech Ozobranchus shipleyi.

I. Occurrence of the Parasite.

Nicoria trijuga occurs in very large numbers all over Ceylon. It generally frequents ponds, lakes, and rivers, but specimens are sometimes found living a semi-terrestrial existence in places removed from water. The tortoises which have adopted the drier habitat occasionally show ticks, but I have never found them infected with the hæmogregarine. I did not, however, examine a sufficiently large number of individuals to be able to draw the conclusion that the dry-dwelling tortoises are never infected.

The Nicorias from the usual aquatic habitat are very often infected with the hæmogregarine. It does not seem to produce any pathogenic effects even when present in large numbers. No other blood-parasites were ever observed in association with the hæmogregarine. The intestinal parasites were not investigated, but it may be noted in passing that a Bodo-like flagellate was found on two occasions in the gall-bladder.

The only ectoparasites present were ticks on dry-land tortoises and leeches on the water-dwelling tortoises. The leeches belonged to a species of Ozobranchus; only once was an isolated Glossiphonia found upon a Nicoria. I found that tortoises from all parts of Ceylon showed the hæmogregarine. I never, however, investigated individuals from more than an elevation of 1500 feet. Generally speaking I found the up-country reptiles were free from blood-parasites.

II. Brief Summary of the Life-history.

For the sake of clearness it is, I think, advisable to give a brief account of the life-history of the form under discussion, in so far as it has been made out, before treating the various points in detail.

The hæmogregarine in the blood of the tortoise shows the usual two types, a bean-shaped and a recurved type. Certain of the bean-shaped individuals, namely, the large forms with a nucleus in which the chromatin is rather loosely arranged, give rise by a process of schizogony in the lung to a great number (about seventy) of large merozoites. Another type of schizogony is found in the circulating blood-corpuscles, and arises also from bean-shaped individuals. This results in the formation of a small number (six to eight) of merozoites of quite small dimensions. It appears that the form which gives rise to this second type of merozoite is itself derived from the schizogony in the lung. It is probable that the small merozoites give rise to the gametocytes. The reason for this assumption is given in another part of the paper. When the hæmogregarines are taken into the crop of the leech, Ozobranchus shipleyi, together with the blood of the tortoise, certain of the hæmogregarines pass into the intestine, and are there found as motile vermicules. They penetrate into the intestinal wall, where the differentiation of the hitherto indistinguishable gametes takes place, culminating in a process suggesting anisogamous conjugation. The zygote breaks up to form eight sporozoites, which pass through the intestinal wall into the blood-spaces. The hæmogregarine is probably passed into the blood of the Nicoria through the contamination of the wound by the leech while feeding.

III. Phases of the Hæmogregarine in the Blood of the Tortoise.

In the living state the hæmogregarine may easily be distinguished as a clear sausage-shaped inclusion in the red blood-corpuscles. The protoplasm is slightly more granular at one end than the other, and the nucleus can be seen as a sharply defined clear area. The parasites do not show any sign of movement when they are observed upon a sealed slide, but free vermicules are very occasionally found in serous blood that has been allowed to stand exposed in the air. The addition of salt-solution to the blood sometimes causes the hæmogregarines to quit the corpuscle, but never in large numbers. Altogether, it may be said that H. nicoriæ shows far less tendency to become motile in the blood than the majority of the species of hæmogregarines.

The greater part of the blood-films made were preserved by the drying method and stained with Giemsa; a few were, however, fixed while still wet in sublimate-acetic, and treated by wet methods throughout. These wet films were stained with iron hæmatoxylin, and it has been clearly shown that wet fixation followed by hæmatoxylin, hæmalum, or other suitable stain, gives far truer pictures than those obtained by the Giemsa method. All the detail of structure, etc., described were worked out on the wet films.

Parts of the various organs, such as the spleen, liver, and lungs, were also preserved (in Flemming, corrosive-acetic, and Bles's fluid) and sections made. Bles's fluid was found to give an exceedingly good fixation of the blood-corpuscles and of the parasites they contained, especially in the tissue from the lung. When stained with hæmalum a very clear and precise picture was obtained, and the results derived from a study of the films could thus be corroborated and criticised by means of the section material.

In the stained films it can be seen that the parasite is surrounded by a delicate sheath or capsule. The nature of this capsule shows the greatest possible variation in different members of the genus Hæmogregarina. In some species it is a thick refractile envelope, which opens to let out the enclosed parasite when the motile phase is adopted. Even when the capsule is more delicate it is often capable of persisting for a time after the hæmogregarine has escaped. This has been observed by many workers; Castellani and Willey (**4**) have shown it in H. mirabilis, Dobell, in a form from Boa constrictor (**6**); I have myself seen the same thing in H. triedrus. In H. nicoriæ the capsule is rather difficult to demonstrate; iron-hæmatoxylin films or those counter-stained with eosin are the best for this purpose. The capsule never persists after the parasite has escaped, in fact it seems that in this case the envelope may be said to disintegrate rather than to be shed in the usual way. The capsule is to be seen quite clearly in the live state in individuals from the crop of the leech, especially at the time when the blood-corpuscle has been already digested away, but before the parasite has passed down to the intestine, where it becomes motile.

The protoplasm is delicately alveolar, and is sometimes slightly granular; chromatoid particles outside the nucleus are very rare, and this form does not show the curious eosinophile inclusions found, for instance, in H. vittatæ (**11**). The nucleus consists, as a general rule, of a number of isolated chromatin granules arranged, often rather symmetrically, round a small central body (see figs. 1–3). The peripheral grains of chromatin may be connected by strands with the central granule. This central granule cannot be called a karyosome in anything approaching the same sense in which this word is applied in protozoan literature generally. In the nucleus of this hæmogregarine it is only the position that marks off the central body from the peripheral chromatin granules; it is in no way distinguished from them in size or staining reaction, and in those cases where the chromatin granules are less regularly arranged (fig. 9A) it is quite impossible to pick it out with certainty. Nevertheless, it appears to me to be of a different nature from the other nuclear elements, in so far that, in the very primitive nuclear division, it seems to form a kind of centrodesmose. Not infrequently the peripheral chromatin granules are joined to one another, a chromatin ring being thus formed all round (see figs. 2, 5, and 8). It must not be supposed that this chromatic ring is truly a nuclear membrane; it takes the chromatin stains deeply, and assumes a bright red colour with Twort's stain. It is thus in sharp contrast to the green membrane found by this method round the nucleus in, for instance, some trypanosomes and certain amœbæ. I am inclined to think that it is simply formed by the running together of the grains of chromatin. Forms are sometimes found in the blood of the tortoise which show the chromatin arranged in an inner and outer ring; this type is shown in fig. 5. Finally, forms are also seen in which the chromatin is in the shape of a large number of irregularly disposed granules, which may at times give the appearance of a kind of reticulum (figs. 6, 17, and 18). In H. nicoriæ, as in almost all the species known, there are, in addition to the young forms, two types in the blood of the vertebrate host, the one a bean-shaped organism with an approximately central nucleus, the other a long recurved creature with its nucleus situated in the broader limb near the bend (see figs. 1, 3, 8, 9, 17 and 18). The bean-shaped form is always present in far greater numbers than the fully developed vermiform individuals, but specimens are very common where the more slender recurved limb is only about half as long as the broad limb (figs. 2 and 9A). This is one of the points in which the wet fixation method is so much superior to the dried films. In the latter the great majority of these specimens, where the recurved limb is shorter than the broad one, appear simply to be bean-shaped, the drying having artificially obscured the recurved limb. There are no very marked or constant nuclear differences in these types; it may, however, be observed that generally speaking the larger bean-shaped forms show the more scattered arrangement of the chromatin. The smaller bean-shaped individuals and the half recurved creatures have usually the more symmetrical circular type of nucleus, while the large vermiform specimens have a slightly elongated nucleus, with a tendency for the chromatin masses to run together at their edge. A glance at the figures will make these points clear.

Two main theories as to the significance of the bean-shaped and vermiform (fully recurved) creatures have been put forward: (1) That the bean-shaped individuals are macrogametes or macrogametocytes, and the recurved ones microgametes or microgametocytes. These two different types or their immediate derivatives are by this view expected to conjugate in the intermediate host and give rise to the sexual cycle. (2) The second view considers that the bean-shaped creatures are responsible for the endogenous cycle within the vertebrate, while the recurved vermiform type carries on the life-history in the intermediate host (**15**). It appears that in H. nicoriæ, at all events, the schizonts (in both types of schizogony) are bean-shaped when they enter upon the process of schizogony (see figs. 10 and 19). I am inclined to think, however, that too much importance has been attached to the difference in shape between the recurved and bean-shaped individuals. The recurving is an appearance caused by the growth in length of the parasite inside its capsule, and there seems to be evidence (fig. 8A) which goes to show that the recurved part is capable of being reabsorbed as the parasite increases in width. It is therefore not improbable that certain of the schizonts are really derived from the vermiform individuals.

Although doubly and trebly infected corpuscles are to be seen, I have never come across any trace of binary fission nor of any process that could reasonably be interpreted as conjugation within the corpuscle. Hahn (**8**) has recently described this process, but I have not been able to corroborate his results.

Schizogony.—Two quite different types of schizogony occur in the vertebrate host. The one takes place in the lung, each schizont giving rise to a very large number (about seventy) of large merozoites. The other takes place in the circulating blood-corpuscle, each schizont producing six to eight quite small merozoites.

Schizogony in the Lung.—The first stage is shown in fig. 19, and is from a section of the lung; it represents a bean-shaped hæmogregarine, rather larger in size than those found in the blood-stream. There is a delicate envelope round the creature, the protoplasm is rather granular, and there is a single nucleus with the chromatin arranged in small irregular grains. The hæmogregarine is not contained in a blood-corpuscle, but is apparently lying free in a capillary of the lung. The schizont now increases immensely in size, and the nucleus multiplies by successive divisions. The mitosis is of a very simple type; the amount of chromatin seems to augment by division of the granules, the nucleus becomes slightly elongated, the central body divides, and the strand of staining material which connects them appears to play the rôle of a simple spindle. The chromatin granules now become loosely grouped about each new central body, and the connecting strand disappears. From the scarcity of division-figures one is inclined to think that this primitive mitosis must take place very rapidly (figs. 16, 21).

During these processes of growth and nuclear multiplication the shape of the body is maintained, and there results a very large bean-shaped or sausage-shaped organism surrounded by a membrane. It is circular in section (figs. 21–23), and contains a large number of nuclei; I have counted about seventy, but the number appears to vary. A point of some interest is that very little, if any, diminution takes place in the size of the nuclei; it will be observed, also, in the figures that they are evenly distributed through the cell-body and not arranged at the periphery.

The protoplasm finally segregates round the nuclei, and there are formed a corresponding number of merozoites, which still lie within the envelope. They are presently set free as sausage-shaped hæmogregarines of 6 to 7·5 μ in length, that is to say, only little below the average size (8 to 10 μ)[1] of the hæmogregarines seen in the blood. They have usually rather regular nuclei of the rounded or slightly elongated type.

Schizogony in the Blood-corpuscle (figs. 10–16).—In the blood of practically all the infected tortoises examined multinucleate hæmogregarines were found in greater or less numbers. These forms may show any number of nuclei up to eight; generally, however, they do not show more than six. From a study of the early binucleate phases it is clear that these specimens arise from bean-shaped hæmogregarines (figs.

10 and 11).[1] The parasite remains inside the blood-corpuscle (fig. 11 is a case where the creature has been liberated mechanically in the making of the film), and does not undergo any increase in size. Finally, the protoplasm segregates round the small, slightly elongated nuclei, and a corresponding number of little falciform merozoites are formed inside the original envelope (figs. 14 and 15). This stage is rather difficult to find and must be of short duration, as it is somewhat rare in comparison with the number of multinucleate creatures to be found in the blood. In the cases I have found the number of merozoites is six, but I should expect that eight may sometimes be formed, as rare stages with more than six nuclei are to be seen (see fig. 13). Schizogony stages of this type occur in blood from any part of the tortoise. The merozoites, which are much smaller (4μ) than those formed in the lung, finally escape and penetrate into another blood-corpuscle, where they proceed to grow. It is unfortunately almost impossible to trace the subsequent career of these young forms with any satisfying measure of certainty. There are, practically speaking, no distinctive features to lay hold of, and once they have increased in size there is nothing to distinguish them from other forms. The impression I have gained in my attempts to follow their development is that they grow into a compact bean-shaped creature of no great size (see figs. 4 and 7). The nucleus is inclined to stain deeply, and is composed of separate granules, which may be arranged irregularly or in a circle—the latter is on the whole the more common. Beyond this point I have not been able to trace these forms; I was always working with natural infections, which appeared to be of a chronic

[1] The question arises as to whether these bean-shaped forms which give rise to the schizogony in the peripheral blood are derived from the vermiform type. The evidence to be drawn from the infections of H. nicoriæ which I examined is very inconclusive. In H. vittatæ, a form parasitic in the tortoise Emyda vittata, however, the recurved type appears only relatively late in the infection, and I am therefore inclined to think it is associated with the later periods of schizogony and possibly with the process as it occurs in the peripheral blood.

type and generally of long standing. It is obvious that only by following the successive stages of the infection in a previously clean tortoise can points like this be really conclusively determined.

Interpretation of the Two Types of Schizogony.—There are three views which might be put forward in explanation of the facts: (1) That the schizogony in the lung with the large merozoites gives rise to the female gametes, and the schizogony in the blood-corpuscles to male gametes. This view is, I think, inadmissible, as it is very unlikely that the small male gametes should be produced in such small numbers, namely six to eight to one parent individual, while the female gametes are produced in large numbers—about seventy to one parent individual.

(2) The second, and I think more probable, explanation is that the schizogony in the lung is the endogenous asexual multiplication, and that certain of the merozoites thus formed proceed in turn to form gametocytes by the schizogony in the blood-stream.

(3) A third quite plausible explanation is that the schizogony in the lung is brought about by the newly injected parasite—that is to say, it is the first activity of the hæmogregarine upon arriving in the vertebrate host. Miller's (**9**) account of Hepatozoon perniciosum, Chagas' (**5**) work on Schizotrypanum, and Aragao's (**1**) on Hæmoproteus columbæ furnish parallels for such an interpretation. On this view the schizogony in the blood-corpuscle would be the later, and, so to speak, chronic process of multiplication, which would at some period culminate in gamete formation.

I think the evidence is strongest in support of the second view (2) put forward, namely that the schizogony in the lung is the asexual multiplication, and that in the blood gamete-formation. A somewhat important point against view (3) is the fact that the schizont in the lung does not appear to penetrate a lung-cell, which one would expect it to do did it arrive in the lung as a free vermicule (sporozoite). Moreover the possession of an envelope in so early a stage as that shown in fig. 19 strongly suggests that it has had an endo-corpuscular existence. I have never seen any sign of the parasite reaching the lung by being engulfed by leucocytes, and I am therefore inclined to think that the schizonts in the lung must have come from the blood-corpuscles.

IV. Stages in the Leech.

Before giving an account of the stages of Hæmogregarina nicoriæ observed in the leech, it will, I think, be well to describe the more important features of the leech itself.

The form in question belongs to the Rhynchobdellid genus Ozobranchus. Mr. W. A. Harding, to whom the leech was sent for identification, found that it belonged to a new species, and called it Ozobranchus shipleyi. It is a small aquatic form carrying a row of feathery gills on each side of its body. The creature rarely reaches more than about one third of an inch in length even when fully extended. Generally speaking, it is found attached to the tortoise at the back of the neck, round the sockets of the limbs, and more rarely upon the ventral side near the throat. The leeches have a tendency to assemble together in groups—a habit they preserve even when kept in a glass dish. The gills of the Ozobranchus are kept in constant motion, and the animal dies if left out of water for any length of time. I was not very successful in getting the leeches to live for long in captivity, nor was I able to discover exactly what was amiss in the conditions to which they were exposed. Possibly the smaller quantity of water rose to too high a temperature. Leeches are usually very hardy and live well in captivity. I had no difficulty in keeping Pœcilobdella alive in Ceylon for months. I have often observed, however, that newly fed specimens are much less resistant than fasting individuals, and this seems true of a number of different species of leech. Almost all the Ozobranchus I got were either in the act of feeding or newly fed, and therefore in the least favourable condition. This leech seems to show a much closer adaptation to its host than generally obtains among the group. Thus it was never found upon Emyda (the milk tortoise) living in the same lake with the Nicoria, nor upon the siluroid fish Saccobranchus, nor upon the water-snakes which shared the same habitat. Even in an area so restricted as a well, these leeches were only found to infest the Nicoria. Moreover, Ozobranchus lays its eggs upon the carapace of the tortoise; they are of a dark brown colour, closely resembling that of the tortoise, and are so firmly cemented on that it requires a knife or some fairly sharp instrument to detach them. It appears that the leeches move readily enough from one tortoise to another, but it is difficult to make out exactly how they are adapted to the terrestrial night-wandering of their host. The Nicoria spends all the day sleeping in the water and comes to land to prowl around at night, so most likely the leeches feed during the day and drop off at night. Generally speaking I got more leeches from nicoria caught in the evening, but there were, however, some exceptions to this; presumably these were cases where the tortoise had spent the night either in the water or in a damp place. Ozobranchus is capable of executing rather feeble swimming movements, and, in addition, can creep around upon its suckers in the usual way. The time taken to digest a meal seems to vary from about three to seven days, according to the size of the leech.

In Ozobranchus shipleyi the proboscis leads into the crop, which is a wide, very extensible sac dividing into two large lobes at its lower end. The intestine opens from the crop at the point where the division takes place. The upper end of the intestine, which is rather wide, shows four long diverticula on each side (see fig. in text, p. 753). This wide part of the intestine terminates in a kind of chamber which opens by a narrow communication into a simple coiled tube, which leads to the exterior at the anus. For some reason the most infected part of the gut wall is almost always this chamber at the end of the wide intestine. The accompanying diagram, which was made from reconstructions of sections by the glass-plate method, shows the relations of the various parts of the alimentary tract. The cells lining the intestine are very large and richly ciliated; their protoplasm has a strong affinity for all nuclear stains, including the red element in Twort's stain. The nuclei are very large and reticulate, often showing several karyosomes.

The stages of the parasite in the leech had to be studied for the most part upon section material; sublimate acetic and Flemming's fluid were the fixatives used. The leeches were usually placed between two slides, so as to prevent undue

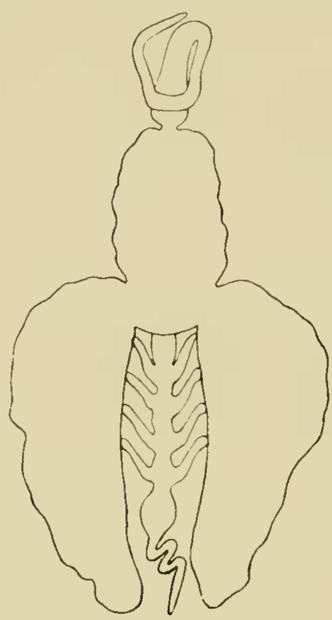

Diagram of the alimentary tract of *Ozobranchus shipleyi*.

retraction. As regards staining, Delafield's hæmatoxylin, Twort's stain, thionin, methyl-blue eosin, and Mayer's hæmalum were all used with good effect, hæmalum and Delafield being the most generally useful. Heidenhain's iron-hæmatoxylin was quite impossible, as it darkened the whole intestinal region so intensely that, long before that region was sufficiently colourised, the remainder of the section was completely bleached.

I am indebted to Mr. Peter Jamieson for the skill with which he has cut the many sections required.

I may mention in passing that the intestine of the leech is, as a general rule, extraordinarily free from bacteria, schizomycetes, etc., and although a total of about 150 leeches were examined, I never found them to contain any flagellates, or, indeed, any protozoan parasites other than the hæmogregarine. A large number of observations upon live material from the leeches were made in the hope that the sequence of the processes might be followed by direct observation, but this proved to be impossible, as the development occurs in the tissues of the leech.

The blood upon being taken up by the leech is stored in the large crop, where the blood-corpuscles undergo a gradual degeneration. The blood passes in small quantities into the intestine, where it is digested and absorbed. Blood-corpuscles are never found, nor even their nuclei, in a recognisable state in the intestine, and this holds good even in the case of a newly fed leech. A large number of live observations were made, but no motile hæmogregarines were ever found in the crop. This particular hæmogregarine appears to be digested out of the corpuscle (figs. 25 and 26), and only to become motile when it passes into the intestine. I am persuaded that this cannot be universal amongst hæmogregarines; so many species react almost instantly to the mere shedding of the blood that I expect in other cases the parasites will be found to become motile at once upon being taken into the intermediate host. Motile hæmogregarines are to be found in the intestine at intervals all through the digestion, but except in cases where the blood is very rich in parasites, there are never a very large number present at one time. The hæmogregarine never makes any attempt to attack the wall of the crop.

A number of hæmogregarines seem to degenerate in the crop (fig. 27), but degeneration stages are only rarely found in the intestine; it seems to fare with these, as with the blood-corpuscles, that they disintegrate before reaching the intestine. So far as my observation goes, neither the large bean-shaped forms nor the completely recurved individuals are to be recognised in the intestine. The individuals which are met with in this situation have a round or oval nucleus with the chromatin grains fairly regularly arranged (figs. 28–34), and seem, as far as morphological features are concerned, to be the motile phases of such types as are shown in figs. 1–3 and 9A from the blood of the Nicoria, and figs. 21 and 26 from the crop. The protoplasm of the hæmogregarines have very little affinity for most stains, and this is particularly true of the stages in the leech.

The motile creatures carry out movements of flexion and also of contraction and extension; in addition to this they can glide by means of very shallow undulations passing down the body. This constricting motion, as in analysis it really is, is most strikingly seen in H. leschenaultii (a hæmogregarine from Hemidactylus leschenaultii), but the difference is purely one of degree.

The faculty of contracting and extending the whole body shown by the motile forms of H. nicoriæ is a disturbing factor when an attempt is being made to divide the parasites into different categories. After much searching, I have come to the conclusion that the only distinction between the parasites while still in the lumen is one of size, and I consider this to have practically no value when one remembers the capacity of the creature for stretching, and the great difficulty in getting a correct idea of bulk in an animal of this type. The drawings have been made from sections, and here one has the additional danger of not always getting the animal in a perfectly horizontal position.

It was noticed not infrequently in the live specimens from the intestine that two equal individuals ranged themselves side by side, but complete fusion was never observed. In the sections this association in couples was again found (see fig. 35), and the individuals showed no differentiation. Here, also, stages indicating complete fusion were not seen; only the two cases figured (figs. 36 and 37) were observed, and as both these are cut obliquely they are not particularly convincing. I therefore think that if appearances such as those shown in figs. 35–37 relate to conjugation at all, they are only instances of (perhaps precocious) association. After a time (figs. 38–41) the hæmogregarines penetrate the intestinal wall, where appearances quite different from those just described suggest conjugation of a type closely resembling that found by Siedlecki (**14**) in Adelea ovata, and by Perez (**10**) in Adelea mesnili. Fig. 44 gives a picture of an early stage; the macrogameta has become differentiated as a large rounded organism with a nucleus in which the chromatin is beginning to form a rather diffuse mass instead of being arranged in the definite granules seen in the motile phase. The nucleus of the microgametocyte is very compact, and stains deeply, the protoplasm has not fused with that of the microgamete, nor does it appear to do so subsequently.

From appearances such as fig. 45, the microgametocyte nucleus seems to divide into three or four, of which two or three, as the case may be, remain outside and degenerate; they sometimes persist for a long time, and are to be seen forming a dense mass of chromatin on the edge of the sporocyst (see figs. 49, 51, 52). The division of the nucleus of the microgametocyte into four is probably the more normal condition, the cases where three are formed being most likely due to the suppression of one of the divisions. One of the four microgamete nuclei thus formed appears to pass into the protoplasm of the macrogamete; unfortunately quite clear pictures of the fusion of the gamete nuclei and the first division of the zygote nucleus were not found. Fig. 47 B shows a condition suggesting the latter stage, but in view of certain reactions on the part of the host-cell to be noted later, I do not feel perfect confidence in this interpretation.

It is quite impossible to pass over these appearances without noting their very probable significance as conjugation and their close resemblance to the fertilisation of Adelea; at the same time I am fully aware of important gaps in the series. Great caution is required in interpreting these appearances, as degenerating hæmogregarines are occasionally found in the gut wall. Moreover, the host-cell seems sometimes in strong infections to react to the presence of the parasite by the formation of internal masses resembling the mucoid globules described by Leger and Duboscq (8 A).

Formation of Sporozoites.

The further development of the parasite culminates in the formation of eight sporozoites. A membrane is secreted round the protoplasm, forming a kind of cyst-wall, but it appears to be thin and not very resistant. Fig. 46 shows an early stage where there are only two nuclei present. Subsequent divisions occur, and appearances such as fig. 48 are produced, where the larger nucleus at one end of the creature is preparing for division. Finally (see figs. 49–53), the protoplasm segregates round the nuclei, and there are produced eight individuals; these when fully developed show considerable resemblance to the free motile forms found in the lumen of the intestine, and are of much the same size. The sporozoites are set free in the wall and pass out into the blood-spaces (see fig. 47 c, 54–56), where they can be distinguished from the corpuscles of the leech by their shape and characteristic nuclear appearance.

There is a well-marked correlation between the processes of digestion in the leech and the condition of the parasite. In a recently fed leech the free motile forms are numerous in the intestine but no multiplicative stages are to be seen in the wall. Later on the hæmogregarines have penetrated the wall, but only the earlier stages are present. Still later ripe cysts with fully formed sporozoites are found in considerable numbers in good infections. Quite late towards the end of digestion, when the crop is empty, the sporozoites have for the most part escaped into the blood-spaces, and the intestinal wall is once more almost free from parasites.

I have not been able to carry my investigations beyond this point, and cannot say by what means the hæmogregarines are passed back into the blood of the tortoise. In spite of much searching I have never found motile stages of the parasite in the proboscis, nor do they appear in this region in the sections.

V. General Remarks and Conclusions.

When the foregoing account was all but complete, I received Dr. Reichenow's (**12**) interesting preliminary note on H. stepanovi. The results I have obtained coincide in all essential points with those of Reichenow, and the evidence he has obtained upon the question of conjugation is much more conclusive than that brought forward by myself, as he has figured the first two divisions of the zygote-nucleus. The type of conjugation is clearly the same in the two cases. The only point of divergence in the two life-cycles is the schizogony in the vertebrate host; in H. stepanovi this takes place in the bone-marrow and always occurs inside the blood-corpuscle, the number of merozoites not exceeding twenty-four. This difference is the main justification for preserving the species name of H. nicoriæ.

There is scarcely a single point in the development of H. stepanovi as described by Siegel (**13**) which is in agreement with the results obtained by Reichenow, or with what I have myself observed in H. nicoriæ. I have never seen the formation of the minute microgametes, nor the sporulating stages in the blood-spaces of the leech, nor the worm-like sporozoites which he describes. It would appear that this worker must have been dealing with conditions differing widely from those presented by the leeches I examined.

It will be observed that the life-cycle of H. nicoriæ differs in one or two points from that of Hepatozoon perniciosum, the hæmogregarine of the rat, described very completely by Miller. The most important divergence occurs in connection with conjugation and the formation of sporoblasts, which in turn produce sporozoites. The sporozoites never become motile in the mite, and the parasite returns to the rat by way of the alimentary tract when the rat eats the mite.

The life-cycle of H. nicoriæ at once recalls the processes observed in Coccidia, but there are two points of difference which are, I think, important as diagnostic characters. Firstly, at no stage does H. nicoriæ show in its nucleus the karyosome so characteristic of the coccidia; secondly, the sporozoites are not enclosed in a resistant cyst, and become motile within a relatively short time after they are formed without the stimulus of transference to another host-individual. In all the coccidia hitherto described the sporozoites remain dormant, until by one means or another they pass to the exterior, and are taken up by another individual of suitable species where the sporozoites are set free. As regards the question as to whether the stages in the leech might not belong to an independent parasite, and have no connection with H. nicoriæ, the following points may be urged: The close correspondence between the stage of digestion and the development of the parasite, the strong morphological resemblance between such stages as those figured in figs. 1, 2, 3, 9A, 25, 26, 28–34, 38–41, 51, 54–56, derived respectively from the blood of the tortoise and different parts of the leech, and the apparent absence of the parasite in leeches taken from uninfected tortoises. Lastly, on the hypothesis that the stages in the leech are independent of those in the tortoise, the only other group in which the forms from the leech could be placed is that of the Coccidia. The points of divergence noted in the preceding paragraph are, I think, sufficiently important to distinguish them from any form belonging to that group. The point is, of course, one which could be determined experimentally when suitable material is available.

Lister Institute,
April, 1910.

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EXPLANATION OF PLATES 32–41,

Illustrating Miss Muriel Robertson's paper on "Studies on Ceylon Hæmatozoa."

[The figures are all drawn with the Abbé camera at a uniform magnification of 2400 diameters.]

Figs. 1–24 represent stages from the vertebrate host Nicoria trijuga. Figs. 1–15 (with the exception of fig. 9) are stages from the blood treated by the wet method throughout and stained with Heidenhain's iron-hæmatoxylin. Figs. 16–24 are from sections of the lung stained with Mayer's hæmalum.

Fig. 1.—Bean-shaped hæmogregarine with circular type of nucleus.

Fig. 2.—Half-recurved specimen with circular nucleus; the chromatin is symmetrically arranged and the central granule is visible.

Fig. 3.—Bean-shaped specimen, the stain further extracted.

Fig. 4.—Small form derived from schizogony in the blood.

Fig. 5.—Bean-shaped specimen showing the chromatin in the nucleus arranged in two rings, one within the other. This creature has been set free mechanically in the making of the film.

Fig. 6.—Bean-shaped specimen with reticulate nucleus.

Fig. 7.—Small compact specimen.

Figs. 8 and 9.—Fully recurved (vermiform) specimens. Fig. 9 is from a dried film stained with Giemsa.

Fig. 8A.—Recurved specimen where the recurved limb is being reabsorbed.

Fig. 9A.—Half-recurved individual, rather broad, and with a large nucleus containing irregularly arranged chromatin.

Figs. 10–13.—Early stages of schizogony in the blood-stream.

Figs. 14 and 15.—Final stages of schizogony in the blood-stream. Fig. 15 has been decolourised to a greater extent than fig. 14; both show six merozoites.

Fig. 16.—Early stage of above type of schizogony from section of the lung; one of the nuclei is undergoing division.

Figs. 17 and 18.—Bean-shaped specimens from section of lung.

Fig. 19.—Earliest stage of schizogony in the lung.

Fig. 20.—Slightly later stage of schizogony; the schizont has increased in size and the nucleus has divided.

Fig. 21.—Still later stage; some of the nuclei appear to be preparing for division.

Fig. 22.—Multinucleate schizont cut across in section.

Fig. 23.—Late stage of schizogony in lung, the protoplasm beginning to segregate round the nuclei.

Fig. 24.—Fully formed merozoites; only a very few of the total number formed are shown in the section.

Figs. 25—56 represent stages in the leech Ozobranchus shipleyi Harding.

Figs. 25 and 26.—Non-motile stages from the crop.

Fig. 27.—Degenerating stage from the crop.

Figs. 28–32.—Free motile stages in the lumen of the intestine of the leech.

Figs. 33–37.—Association in the lumen of the intestine.

Figs. 38–41.—Early stages in the cells of the intestinal wall.

Fig. 42.—Precocious differentiation of microgamete.

Fig. 43.—Early stage of macrogamete.

Figs. 44 and 45.—Stages suggesting conjugation. In fig. 44 the microgametocyte is lying closely applied to the macrogamete. In fig. 45 the microgametocyte appears to be giving rise to the microgamete nuclei, one of which will fuse with the nucleus of the macrogamete.

Fig. 46.—Early stage of sporocyst showing two nuclei.

Fig. 47.—(A) Stage apparently representing a zygote; (B) stage showing what appears to be the first division of the zygote nucleus; (C) free sporozoites in the wall of the intestine.

Fig. 48.—Stage of above showing five nuclei, of which one is preparing to divide.

Fig. 49.—Sporocyst with eight nuclei; the protoplasm has not yet divided up; the rejected microgamete nuclei are still visible.

Figs. 50–53.—Sporocysts showing sporozoites.

Figs. 54 and 55.—Motile sporozoites escaping through the cells of the intestinal wall.

Fig. 56.—Sporozoite in blood-space of the leech.